IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF :

KAZUMITSU NAKATSUKA : GROUP ART UNIT: 1615

SERIAL NO: 10/030,422

FILED: January 10, 2002 : EXAMINER: Liliana Di Nola-Baron

FOR : ANTIMICROBIAL COMPOSITION

DECLARATION UNDER 37 C.F.R.1.132

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

SIR:

Now comes Kazumitsu NAKATSUKA who deposes and states that:

1. I am an inventor of the invention claimed in the above identified application.

2. I graduated from the faculty of pharmaceutical sciences, Okayama University in 1993, and I have been a research scientist since April 1993 in Kuraray Co., Ltd., working on dental materials.

3. I have studied the official Action of August 12, 2003.

4. I conducted under my supervision and direction the following experiments in order to make the present invention clear.

5. In these experiments, antibacterial property, adhesiveness and storage stability were tested according to the following antibacterial test, bonding test and storage stability test, respectively.

[Antibacterial Test]

1 g of bovine dentin powder that had been previously sterilized and dried, 0.5 ml of the antibacterial composition obtained in the following Comparative Example, and 0.5 ml of aqueous 50% HEMA solution were put into a sample tube, stirred for 10 minutes, and then centrifuged to collect the upper liquid phase. This is a 50% sample liquid. The 50% sample liquid was diluted with sterilized water to prepare different samples having a concentration of 20%, 10%, 5%, 2% and 1%. On the other hand, cells of *Streptococcus mutans* (IFO13955) that had been pre-incubated for 18 hours in a liquid brain heart infusion (BHI) medium (from Nippon Pharmaceutical) were diluted with germ-free water to prepare a cell dilution having a cell concentration of 2×10^6 (CFU/ml). The samples having

different concentrations as above were tested with the cell dilution for the antibacterial property.

Concretely, 100 μl of each sample and 100 μl of the cell dilution were rapidly mixed on a micro-plate. After 20 seconds, the resulting mixture was diluted with BHI to 1/1000. 100 μl of the 1/1000 dilution was metered, applied onto a BHI-agar medium (from Nippon Pharmaceutical) plate that had been separately prepared, and uniformly spread thereover with a Conradi rod. With that, the plate was put in a thermostat at 37℃ and the cells on the plate were aerobically incubated therein for 48 hours. For control, germ-free water alone not containing the antibacterial composition was tested in the same manner as above. The number of colonies formed in the BHI-agar medium was counted, and the cell death percentage was calculated according to the following equation:

Cell Death Percentage (%)={[number of colonies (in control)-number of colonies (in antibacterial composition-containing sample)]/number of colonies (in control)} x 100

[Bonding Test]

A bovine anterior tooth was polished in wet with #1000 Silicon Carbide Abrasive Paper (from Nippon Abrasive Paper) to make its surface smooth, then its enamel or dentin was exposed out, and water existing on its surface was blown off with a dental air syringe. An adhesive tape (thickness: about 150 microns) with a hole having a diameter of 3 mm was stuck on the surface of the exposed enamel or dentin. The antibacterial composition obtained in the following Comparative Example was applied to the holed area with a brush, then left as such for 30 seconds, and dried with an air syringe until the antibacterial compositions was no more fluid. Next, a photopolymerizable, dental bonding material "Clearfilmegabond" (from Kuraray) was applied over it also with a brush to form thereon a layer having a thickness of about 100 μm. With that, this was exposed to light for 10 seconds and cured, for which used was a dental light emitter "Litel II" (from Gunma Ushio Electric). Next, a commercially-available, photopolymerizable dental composite resin, "Clearfill AP-X" (from Kuraray) was put on it, covered with a film of Eval®. (from Kuraray), and pressed against a glass slide superposed thereon. In that condition, this was exposed to light for 40 seconds and cured, for which was used the same light emitter as above.

A stainless steel rod was attached to the cured surface with a

commercially-available dental resin cement, "Panavia 21" (from Kuraray) being disposed therebetween. After left as such for 30 minutes, the test piece was dipped in water at 37℃ for 24 hours, and then its bonding strength was measured. For the measurement, used was a universal tester (from Instron). At a cross head speed of 2 mm/min, the tensile bonding strength of the test piece was measured. Eight test pieces were prepared and tested under the same condition for their bonding strength, and their data were averaged.

[Storage Stability Test]

(1) Discoloration Test:

The antibacterial composition obtained in the following Comparative Example was stored in a thermostat at 50℃ for 1 month. 200 μl of it was put into a colorless transparent glass chamber having a diameter of 1.4 mm, a depth of 2 mm and a thickness of 1 mm, and its values L* and b* were measured with a colorimeter (from Nippon Denshoku Kogyo). Not stored, a fresh sample of the composition was also measured in the same manner. From the data, obtained were ΔL* and Δb*. In addition, the stored sample was visually checked for discoloration.

(2) Bonding Test:

The antibacterial composition obtained in the following Comparative Example was stored in a thermostat at 50℃ for 1 month. In the same bonding test as above, the thus-stored sample was tested for the tensile bonding strength to bovine dentin.

The meanings of the abbreviations used herein are mentioned below.

[Antibacterial salt compound]

MDPB : 12-methacryloyloxydodecylpyridinium bromide

[Acid group-having polymerizable monomer]

MDP : 10- methacryloyloxydecyl dihydrogenphosphate

[Hydrophilic polymerizable monomer]

HEMA: 2-hydroxyethyl methacrylate

[Polymerizable monomers disclosed in U.S. Patent 5,733,949]

MMA : methyl methacrylate

MAA: methyl acrylate

MPS: γ - methacryloyloxypropyltrimethoxysilane

3G: triethyleneglycoldimethacrylate

3GA: triethyleneglycoldiacrylate

NPG: neopenthylglycol- dimethacrylate

NPGA: neopenthylglycol- diacrylate

HD: 1, 6-hexanediol-dimethacrylate

HAD: 1, 6-hexanediol-diacrylate

DD: 1, 10-decandioldimethacrylate

DDA: 1, 10-decandioldiacrylate

BMP: 2, 2' -bis [(methacryloyloxypolyethoxy)phenyl]propane

BAP: 2, 2' -bis [(acryloyloxypolyethoxy)phenyl]propane

Bis-GMA: 2,2'-bis[4-(3-methacryloyloxy-2-hydroxypropoxy)phenyl] propane

Bis- GAA: 2,2'-bis[4-(3-acryloyloxy-2-hydroxypropoxy)phenyl]propane

TMA: trimethylolpropane-trimethacrylate

TAA: trimethylolpropane-triacrylate

Comparative Examples

The antibacterial compositions comprising MDPB (5 parts by weight), MDP (15 parts by weight), HEMA (40 parts by weight), distilled water (40 parts by weight) and an other polymerizable monomer disclosed in U.S. Patent 5,733,949 (3 parts by weight) as shown in Table 1 and 2 were prepared. These compositions were tested for the antibacterial property, the adhesiveness and the storage stability, according to the antibacterial test, the bonding test and the storage stability test mentioned above. The results are given in Table 1 and 2.

Table 1

		Blend Ration (wt.%)						
		Comp.Ex. 1	Comp.Ex. 2	Comp.Ex. 3	Comp.Ex. 4	Comp.Ex. 5	Comp.Ex. 6	Comp.Ex. 7
Antibacterial Composition	MDPB	5	5	5	5	5	5	5
	MDP	15	15	15	15	15	15	15
	HEMA	40	40	40	40	40	40	40
	Distilled water	40	40	40	40	40	40	40
	MMA	3	–	–	–	–	–	–
	MAA	–	3	–	–	–	–	–
	MPS	–	–	3	–	–	–	–
	3G	–	–	–	3	–	–	–
	3GA	–	–	–	–	3	–	–
	NPG	–	–	–	–	–	3	–
	NPGA	–	–	–	–	–	–	3
①Antibacterial Property (Cell Death Percentage : %)								
Concentration	20%	100	100	100	100	100	100	100
	10%	100	100	100	100	100	100	100
	5%	58	50	54	56	57	58	55
	2%	16	13	18	18	13	12	18
	1%	1	7	3	5	6	0	1
②Adhesiveness (Mpa)								
	Bovine enamel	18.1	18.1	18.1	18.1	18.1	18.1	18.1
	Bobine dentin	13.8	14.8	14.5	14.9	14.3	13.9	14.7
③Storage Stability (at 50℃ for 1 month)								
Discoloration	ΔL*	0.6	0.7	0.8	0.7	0.6	0.8	0.7
	Δb*	1.7	1.8	1.6	1.7	1.8	1.6	1.6
	Visual check	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Adhesiveness	Bovine dentin(MPa)	7.2	7.4	8.2	7.6	6.8	8.1	8.5

Table 2

		Blend Ration (wt.%)									
		Comp.Ex. 8	Comp.Ex. 9	Comp.Ex. 10	Comp.Ex. 11	Comp.Ex. 12	Comp.Ex. 13	Comp.Ex. 14	Comp.Ex. 15	Comp.Ex. 16	Comp.Ex. 17
Antibacterial Composition	MDPB	5	5	5	5	5	5	5	5	5	5
	MDP	15	15	15	15	15	15	15	15	15	15
	HEMA	40	40	40	40	40	40	40	40	40	40
	Distilled water	40	40	40	40	40	40	40	40	40	40
	HD	3	–	–	–	–	–	–	–	–	–
	HDA	–	3	–	–	–	–	–	–	–	–
	DD	–	–	3	–	–	–	–	–	–	–
	DDA	–	–	–	3	–	–	–	–	–	–
	BMP	–	–	–	–	3	–	–	–	–	–
	BAP	–	–	–	–	–	3	–	–	–	–
	Bis-GMA	–	–	–	–	–	–	3	–	–	–
	Bis-GAA	–	–	–	–	–	–	–	3	–	–
	TMA	–	–	–	–	–	–	–	–	3	–
	TAA	–	–	–	–	–	–	–	–	–	3
①Antibacterial Property (Cell Death Percentage : %)											
Concentration	20%	100	100	100	100	100	100	100	100	100	100
	10%	100	100	100	100	100	100	100	100	100	100
	5%	100	100	51	53	54	58	55	53	52	59
	2%	20	21	19	11	10	18	16	19	13	12
	1%	1	3	2	4	3	7	6	0	1	0
②Adhesiveness (Mpa)											
	Bovine enamel	18	18.2	17.6	17.5	17.6	18	18.5	17.9	18	18.2
	Bobine dentin	14.2	14.7	13.9	14.4	14.6	14.6	14.8	14.9	14.8	14.5
③Storage Stability (at 50℃ for 1 month)											
Discoloration	ΔL*	0.7	0.7	0.6	0.7	0.9	0.6	0.7	0.8	0.8	0.8
	Δb*	1.8	1.7	1.9	1.8	1.7	1.7	1.6	1.7	1.7	1.6
	Visual check	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless	colorless
Adhesiveness	Bovine dentin(MPa)	8.4	8.6	7.9	7.9	8	8.1	8.5	8	8.3	7

6. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date : January 23, 2004

By: Kazumitsu Nakatsuka

Kazumitsu NAKATSUKA